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## PATENT APPLICATION FULL TEXT AND IMAGE DATABASE



( 1 of 1 )

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**United States Patent Application****20040005678****Kind Code****A1****Keasling, Jay ; et al.****January 8, 2004**

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**Biosynthesis of amorpho-4,11-diene**

### Abstract

Methods for synthesizing amorpho-4,11-diene synthase from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. Amorpho-4,11-diene synthase is then produced using an optimized amorpho-4,11-diene synthase gene. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

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**Douglas;** (*Oakland, CA*) ; **Withers, Sydnor T. III;** (*Richmond, CA*) ; **Newman, Jack;**  
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                         **US**

**Serial No.:**      **411066****Series Code:**    **10****Filed:**            **April 9, 2003****U.S. Current Class:**                    **435/146; 435/193; 435/252.3; 435/320.1; 536/23.2****U.S. Class at Publication:**            **435/146; 435/193; 435/252.3; 435/320.1; 536/23.2****Intern'l Class:**                    **C12P 007/42; C12N 009/10; C07H 021/04; C12N 001/21;**  
   **C12N 015/74**

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### Claims

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L3: Entry 2 of 2

File: USPT

Aug 8, 2000

US-PAT-NO: 6100451

DOCUMENT-IDENTIFIER: US 6100451 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Pathogen-inducible regulatory element

DATE-ISSUED: August 8, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chappell; Joseph	Lexington	KY		
Cornett; Catherine A. G.	Lexington	KY		
Yin; Shaohui	Lexington	KY		

US-CL-CURRENT: 800/298; 435/320.1, 435/419, 435/468, 536/24.1, 800/278, 800/279, 800/301, 800/317, 800/317.3, 800/319

## CLAIMS:

What is claimed is:

1. A recombinant nucleic acid molecule comprising a pathogen- or elicitor-inducible transcriptional regulatory element comprising nucleotides 463-473 of SEQ ID NO:2, nucleotides 406 to 486 of SEQ ID NO:2, nucleotides 463 to 572 of SEQ ID NO:2, nucleotides 371 to 463 of SEQ ID NO:2, or nucleotides 411 to 457 of SEQ ID NO:2.
2. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a gene encoding a terpene cyclase.
3. The nucleic acid molecule of claim 2, wherein said terpene cyclase is a sesquiterpene cyclase.
4. The nucleic acid molecule of claim 3, wherein said transcriptional regulatory element directs expression of an epi-5-aristolochene synthase (EAS).
5. The nucleic acid molecule of claim 4, said nucleic acid molecule comprising the nucleotide sequence shown in FIG. 3A (SEQ ID NO:14) or a pathogen- or elicitor-inducible fragment thereof.
6. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule has the nucleotide sequence shown in FIG. 3A (SEQ ID NO: 14).
7. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a dicot.

8. The nucleic acid molecule of claim 7, wherein said dicot is a member of the Solanaceae.
9. The nucleic acid molecule of claim 8, wherein said Solanaceous plant is a member of the genus *Nicotiana*.
10. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a monocot.
11. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a gymnosperm.
12. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is obtained from a conifer.
13. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is genomic DNA.
14. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is chemically-synthesized DNA.
15. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is a combination of genomic DNA and chemically-synthesized DNA.
16. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is a combination of genomic DNA and cDNA or a combination of genomic DNA, cDNA, and chemically-synthesized DNA.
17. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element increases downstream gene expression in plant tissue in response to an elicitor or a plant pathogen.
18. The nucleic acid molecule of claim 17, wherein said plant pathogen is a fungus.
19. The nucleic acid molecule of claim 18, wherein said fungus is a member of the genus *Phytophthora*.
20. The nucleic acid molecule of claim 17, wherein said plant pathogen is a bacterium.
21. The nucleic acid molecule of claim 20, wherein said bacterium is a member of the genus *Pseudomonas*.
22. The nucleic acid molecule of claim 17, wherein said plant pathogen is a virus.
23. The nucleic acid molecule of claim 22, wherein said virus is tobacco mosaic virus.
24. The nucleic acid molecule of claim 1, wherein said transcriptional regulatory element is induced by an elicitor.

25. The nucleic acid molecule of claim 17, wherein said elicitor is a fungal elicitor.
26. The nucleic acid molecule of claim 17, wherein said elicitor is a bacterial elicitor.
27. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is operably linked to nucleotide sequences encoding a heterologous polypeptide.
28. The nucleic acid molecule of claim 27, wherein said heterologous polypeptide is capable of conferring disease-resistance to a plant.
29. The nucleic acid molecule of claim 28, wherein said heterologous polypeptide is an elicitin.
30. The nucleic acid molecule of claim 28, wherein said elicitin is a fungal elicitin.
31. The nucleic acid molecule of claim 30, said fungal elicitin being from *Phytophthora*.
32. The nucleic acid molecule of claim 31, said elicitin comprising a ParA1 polypeptide.
33. The nucleic acid molecule of claim 29, wherein said elicitin is a bacterial elicitin.
34. The nucleic acid molecule of claim 33, wherein said bacterial elicitin is a harpin.
35. The nucleic acid molecule of claim 27, wherein the expression of said heterologous polypeptide is mediated by one or more external agents.
36. The nucleic acid molecule of claim 27, wherein said nucleic acid molecule expresses said heterologous polypeptide in a cell-specific manner.
37. The nucleic acid molecule of claim 27, wherein said heterologous polypeptide is a pharmaceutical protein.
38. A vector comprising the DNA of claim 1.
39. The vector of claim 38, wherein said vector inducibly expresses a nucleotide sequence in a cell comprising said vector.
40. The vector of claim 39, said nucleotide sequence coding for a heterologous polypeptide.
41. A transgenic plant comprising the nucleic acid molecule of claim 1 integrated into the genome of said plant.
42. A transgenic plant comprising the nucleic acid molecule of claim 27 integrated into the genome of said plant.
43. A seed from the transgenic plant of claim 41.
44. A seed from the transgenic plant of claim 42.

45. A cell from the transgenic plant of claim 41.

46. A cell from the transgenic plant of claim 42.

47. A method of providing disease-resistance to a transgenic plant, said method comprising the steps of:

(a) producing a transgenic plant cell comprising the nucleic acid molecule of claim 28 integrated into the genome of said transgenic plant cell; and

(b) regenerating said transgenic plant from said plant cell wherein the expression of said nucleic acid molecule of claim 28 confers disease-resistance to said transgenic plant.

48. The method of claim 47, wherein said transgenic plant is a dicot.

49. The method of claim 48, wherein said dicot is a member of the Solanaceae.

50. The method of claim 49, wherein said member of the Solanaceae is a member of the genus *Nicotiana*.

51. The method of claim 47, wherein said transgenic plant is a monocot.

52. The method of claim 47, wherein said transgenic plant is a gymnosperm.

53. The method of claim 47, wherein said transgenic plant is a conifer.

54. A method of increasing the transcriptional expression of a downstream DNA sequence in a transgenic plant cell, said method comprising the steps of:

(a) producing a transgenic plant cell comprising the nucleic acid molecule of claim 1 positioned for increasing transcription of a downstream DNA sequence and integrated into the genome of said transgenic plant cell; and

(b) regenerating said transgenic plant from said plant cell.

## WEST Search History

DATE: Wednesday, June 09, 2004

Hide?	Set Name	Query	Hit Count
		<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ</i>	
<input type="checkbox"/>	L3	sesquiterpene cyclase.clm.	2
<input type="checkbox"/>	L2	sesquiterpene cyclase with dna	1
<input type="checkbox"/>	L1	sesquiterpene cyclase	47

END OF SEARCH HISTORY

=> file hcaplus agricola biosis biotechds

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=> s sesquiterpene cyclase and dna

L1 59 SESQUITERPENE CYCLASE AND DNA

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 40 DUP REM L1 (19 DUPLICATES REMOVED)

=> s l2 and 1990-1998/py

L3 19 L2 AND 1990-1998/PY

=> focus l3

PROCESSING COMPLETED FOR L3

L4 19 FOCUS L3 1-

=> s l4 and artemisia annua

L5 0 L4 AND ARTEMISIA ANNUA

=> d l4 1-19 ibib ab

L4 ANSWER 1 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:487012 HCAPLUS

DOCUMENT NUMBER: 123:279727

TITLE: Cloning and bacterial expression of a  
**sesquiterpene cyclase** from  
Hyoscyamus muticus and its molecular comparison to  
related terpene cyclases

AUTHOR(S): Back, Kyoungwhan; Chappell, Joseph

CORPORATE SOURCE: Agron. Dep., Univ. Kentucky, Lexington, KY,  
40546-0091, USA

SOURCE: Journal of Biological Chemistry (1995),  
270(13), 7375-81

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular  
Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Genomic and cDNA clones for vetispiradiene synthase, **sesquiterpene cyclase** found in Hyoscyamus muticus, were isolated using a combination of reverse transcription-polymerase chain reactions and conventional cloning procedures. RNA blot hybridization demonstrated an induction of mRNA consistent with the induction of cyclase enzyme activity in elicitor-treated cells, DNA blot hybridization indicated a gene family of 6 to 8 members, and bacterial expression of 3 cDNA clones indicated that each coded for a vetispiradiene synthase enzyme activity catalyzing the synthesis of a single reaction product. Intron-exon organization of the vetispiradiene synthase gene was identical with that previously described for 5-epi-aristolochene synthase (tobacco

**sesquiterpene cyclase**) and casbene synthase (castor bean diterpene cyclase), and the vetispiradiene synthase amino acid sequence was 77% identical with and 81% similar to the tobacco **sesquiterpene cyclase**. Regions of the vetispiradiene synthase sequence centered around amino acids 60, 100, and 370 were conspicuously different relative to the tobacco **sesquiterpene cyclase**. The sequence similarity between the tobacco and *H. muticus* enzymes is suggested to be reflective of the conservation of several partial reactions common to both enzymes, and the difference may be reflective of a partial reaction unique to each enzyme.

L4 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1997:710590 HCAPLUS  
DOCUMENT NUMBER: 128:71516  
TITLE: Regulation of **sesquiterpene cyclase**  
gene expression. Characterization of an elicitor- and  
pathogen-inducible promoter  
AUTHOR(S): Yin, Shaohui; Mei, Leng; Newman, Jeffrey; Back,  
Kyoungwhan; Chappell, Joe  
CORPORATE SOURCE: Plant Physiology/Biochemistry/Molecular Biology  
Program, University of Kentucky, Lexington, KY,  
40546-0091, USA  
SOURCE: Plant Physiology (1997), 115(2), 437-451  
CODEN: PLPHAY; ISSN: 0032-0889  
PUBLISHER: American Society of Plant Physiologists  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The promoter for a tobacco (*Nicotiana tabacum*) **sesquiterpene cyclase** gene, a key regulatory step in sesquiterpene phytoalexin biosynthesis, has been analyzed. The EAS4 promoter was fused to the .beta.-glucuronidase (GUS) reporter gene, and the temporal and spatial expression patterns of GUS activity were examd. in stably transformed plants and in transient expression assays using electroporated protoplasts of tobacco. No GUS activity was obsd. in any tissues under normal growth conditions. A low level of GUS activity was detected in wounded leaf, root, and stem tissues, whereas a much higher level was obsd. when these tissues were challenged with elicitors or microbial pathogens. The GUS expression pattern directed by the EAS4 promoter was identical to the induction patterns obsd. for the endogenous **sesquiterpene cyclase** genes. Neither exogenous salicylic acid nor Me jasmonate induced GUS expression; and H2O2 induced GUS expression to only a limited extent. Although the EAS4 promoter contains cis-sequences resembling previously identified transcriptional control motifs, other cis-sequences important for quant. and qual. gene expression were identified by deletion and gain-of-function analyses. The EAS4 promoter differs from previously described pathogen-/elicitor-inducible promoters because it only supports inducible gene expression and directs unique spatial expression patterns.

REFERENCE COUNT: 91 THERE ARE 91 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1993:532574 HCAPLUS  
DOCUMENT NUMBER: 119:132574  
TITLE: Gene family for an elicitor-induced  
**sesquiterpene cyclase** in tobacco  
AUTHOR(S): Facchini, Peter J.; Chappell, Joseph  
CORPORATE SOURCE: Agron. Dep., Univ. Kentucky, Lexington, KY,  
40546-0091, USA  
SOURCE: Proceedings of the National Academy of Sciences of the  
United States of America (1992), 89(22),  
11088-92  
CODEN: PNASA6; ISSN: 0027-8424  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The initial step in the conversion of the isoprenoid intermediate farnesyl



diphosphate to the sesquiterpenoid phytoalexin capsidiol in elicitor-treated tobacco tissues is catalyzed by an inducible **sesquiterpene cyclase** [5-epi-aristolochene synthase (EAS)]. Two independent cDNA clones (cEAS1 and cEAS2) encoding EAS were isolated from an elicitor-induced tobacco cDNA library by differential hybridization and subsequently were characterized by hybrid selection-in vitro translation. Insertion of cEAS1, a partial cDNA clone encoding 175 C-terminal amino acids, into an Escherichia coli expression vector resulted in accumulation of a fusion protein immunodetectable with EAS-specific polyclonal antibodies. The cDNA clones were used to isolate two full-length EAS genes that mapped 5 kilobases (kb) apart on one 15-kb genomic clone. The nucleotide sequences of the structural gene components were identical from 388 base pairs (bp) upstream of the transcription initiation site to 40 bp downstream of the translation termination codon, suggesting a relatively recent duplication event. The genes consist of 1479-bp open reading frames, each contg. five introns and specifying 56,828-Da proteins. The N-terminal amino acid sequence deduced from the genomic clones was identical to the first 16 amino acids of the EAS protein identifiable by Edman degradn. RNA blot hybridization with cEAS1 demonstrated a mRNA induction time course consistent with the induction of the EAS mRNA translational activity with max. levels 4-6 h after elicitation. EAS mRNA was not detected in control cells. **DNA** blot-hybridization anal. of genomic **DNA** revealed a copy no. of .apprxq.12-15 for EAS-like genes in the tetraploid tobacco genome. The conservation of a putative allelic prenyl diphosphate binding motif is also discussed.

L4 ANSWER 4 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1998:717424 HCAPLUS

DOCUMENT NUMBER: 130:134851

TITLE: Characterization of the TAC box, a cis-element within an elicitor-inducible **sesquiterpene cyclase** promoter

AUTHOR(S): Newman, Jeffrey D.; Yin, Shaohui; Chappell, Joseph

CORPORATE SOURCE: Plant Physiology and Molecular Biology Program, University of Kentucky, Lexington, KY, 40546-0091, USA

SOURCE: Plant Journal (1998), 16(1), 1-12

CODEN: PLJUED; ISSN: 0960-7412

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The first unique step in the synthesis of the tobacco phytoalexin capsidiol is cyclization of farnesyl pyrophosphate catalyzed by 5-epi-aristolochene synthase (EAS), a **sesquiterpene cyclase**. Earlier work demonstrated that the elicitor-inducibility of this enzyme activity corresponded to the transcriptional activation of at least one gene, EAS4, of a rather complex gene family consisting of >10 members. To investigate the mechanism(s) controlling expression of this gene, fragments of the EAS4 promoter were examd. for binding by proteins in nuclear and whole-cell exts. A strong protein binding site (TAC box; ACTCTACAGTACTC) was identified between -245 and -232 by the electrophoretic mobility shift assay, and DNase I and methylation interference footprinting. Several distinctly migrating bands representing protein-TAC box complexes were also obsd. in the mobility shift assays, and the relative abundance of these bands varied in exts. from cells at different stages of EAS induction. The TAC box binding factor (TacBBF) was purified > 450-fold from crude whole-cell exts. by a combination of **DNA** affinity and cation exchange chromatog. The purified fractions were enriched for polypeptides of 17 and 19 kDa and the **DNA** binding properties of these preps. were characterized. Mutation of 2 bp in the TAC box prevented protein binding in vitro and increased both basal and elicitor-inducible gene expression 2.5-fold in transgenic tobacco plants harboring promoter-GUS fusions, consistent with the notion that this cis-element functions as a silencer or repressor of EAS gene expression.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:547508 HCAPLUS

DOCUMENT NUMBER: 133:160583

TITLE: Plant regulatory elements involved in the hypersensitive response to infection and their uses  
INVENTOR(S): Chappell, Joseph; Cornett, Catherine A. G.; Yin, Shauhui

PATENT ASSIGNEE(S): Board of Trustees of the University of Kentucky, USA  
SOURCE: U.S., 39 pp., Cont.-in-part of U.S. Ser. No. 471,983, abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6100451	A	20000808	US 1995-577483	19951222
US 5981843	A	19991109	US 1995-443639	19950518
CA 2221348	AA	19961121	CA 1996-2221348	19960507 <--
WO 9636697	A1	19961121	WO 1996-US6452	19960507 <--
W:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML			
AU 9657321	A1	19961129	AU 1996-57321	19960507 <--
AU 724614	B2	20000928		
EP 828822	A1	19980318	EP 1996-915576	19960507 <--
R:	BE, DE, FR, GB, IT			
CN 1191565	A	19980826	CN 1996-195657	19960507 <--
JP 11505423	T2	19990521	JP 1996-534883	19960507
ES 2134170	A1	19990916	ES 1997-50002	19960507
ES 2134170	B1	20001201		
SG 74110	A1	20000718	SG 1998-6010	19960507
AP 924	A	20010104	AP 1997-1143	19960507
W:	KE, LS, MW, SD, SZ, UG			
ZA 9603957	A	19961125	ZA 1996-3957	19960517 <--
BR 9602338	A	19980113	BR 1996-2338	19960517 <--
TW 475945	B	20020211	TW 1996-85105864	19960517
US 6605764	B1	20030812	US 1999-435380	19991105

PRIORITY APPLN. INFO.:  
US 1995-443639 A1 19950518  
US 1995-471983 B2 19950606  
US 1995-577483 A 19951222  
WO 1996-US6452 W 19960507

AB Qual. transcriptional regulatory sequences functional in plants, plant tissue and in plant cells for inducible gene expression and quant. transcriptional regulatory sequences for increasing the transcriptional expression of downstream genetic information in plants, plant tissue and plant cells are disclosed. Also disclosed are methods and recombinant DNA mols. for improving the disease resistance of transgenic plants, esp. wherein an inducible promoter controls the expression of a protein capable of evoking the hypersensitive response in a plant. Regulatory elements derived from genes of phytoalexin biosynthesis that are induced as part of the hypersensitive response to infection by plant pathogenic fungi are described for use in increasing plant resistance to infection. The coding and regulatory regions of the tobacco epi-5-aristolochene synthase genes EAS3 and EAS4 genes of tobacco were cloned by std. methods. The promoter regions of these genes were used to drive expression of reporter genes in transgenic tobacco and the minimal

sequence requirements for elicitor induction of EAS4 expression detd. by deletion anal. Expression of the parA1 gene of *Phytophthora parasitica* from the EAS4 promoter in transgenic plants led to increased resistance to infection by *P. parasitica* var. *Nicotianae*.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1991:529134 BIOSIS  
DOCUMENT NUMBER: PREV199192140594; BA92:140594  
TITLE: EXPRESSION OF A FUNGAL **SESQUITERPENE CYCLASE** GENE IN TRANSGENIC TOBACCO.  
AUTHOR(S): HOHN T M [Reprint author]; OHLROGGE J B  
CORPORATE SOURCE: MYCOTOXIN RES UNIT, NATL CENT AGRIC UTILIZATION RES, AGRIC RES SERVICE, US DEP AGRIC, PEORIA, ILL 61604, USA  
SOURCE: Plant Physiology (Rockville), (1991) Vol. 97, No. 1, pp. 460-462.  
CODEN: PLPHAY. ISSN: 0032-0889.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 19 Nov 1991  
Last Updated on STN: 8 Jan 1992

AB The complete coding sequence for the trichodiene synthase gene from *Fusarium sporotrichioides* was introduced into tobacco (*Nicotiana tabacum*) under the regulation of the cauliflower mosaic virus 35S promoter. Expression of trichodiene synthase was demonstrated in the leaves of transformed plants. Leaf homogenates incubated with [3H]farnesyl pyrophosphate produced trichodiene as a major product. Trichodiene was detected in the leaves of a transformed plant at a level of 5 to 10 nanograms per gram fresh weight. The introduction of a fungal **sesquiterpene cyclase** gene into tobacco has resulted in the expression of an active enzyme and the accumulation of low levels of its sesquiterpenoid product.

L4 ANSWER 7 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1993:227090 HCAPLUS  
DOCUMENT NUMBER: 118:227090  
TITLE: Aristolochene synthase. Isolation, characterization, and bacterial expression of a sesquiterpenoid biosynthetic gene (Aril) from *Penicillium roqueforti*  
AUTHOR(S): Proctor, Robert H.; Hohn, Thomas M.  
CORPORATE SOURCE: Mycotoxin Res. Unit, U. S. Dep. Agric., Peoria, IL, 61604, USA  
SOURCE: Journal of Biological Chemistry (1993), 268(6), 4543-8  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Aristolochene is the likely precursor of the sesquiterpenoid toxins produced by a no. of filamentous fungi. One of these, PR-toxin, is produced by *Penicillium roqueforti*. Here the isolation of a gene (Aril) coding for the **sesquiterpene cyclase**, aristolochene synthase (AS), from *P. roqueforti* is reported. Nucleotide sequence anal. of genomic and cDNA clones revealed that the Aril gene contains 2 introns. A Protein A/AS fusion enzyme was expressed in *Escherichia coli* and shown to have **sesquiterpene cyclase** activity. Anal. of the Protein A/AS fusion enzyme reaction mixts. by TLC and gas chromatog./mass spectrometry identified aristolochene as a major product. The Aril gene encodes a polypeptide of mol. wt. 39,200. Expression of Aril occurs in stationary phase cultures of *P. roqueforti* and appears to be transcriptionally regulated.

L4 ANSWER 8 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1993:58175 HCAPLUS

DOCUMENT NUMBER: 118:58175  
TITLE: Overproduction of soluble trichodiene synthase from  
Fusarium sporotrichioides in Escherichia coli  
AUTHOR(S): Cane, David E.; Wu, Zhen; Oliver, John S.; Hohn,  
Thomas M.  
CORPORATE SOURCE: Dep. Chem., Brown Univ., Providence, RI, 02912, USA  
SOURCE: Archives of Biochemistry and Biophysics (1993  
, 300(1), 416-22  
CODEN: ABBIA4; ISSN: 0003-9861  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Trichodiene synthase is a **sesquiterpene cyclase**  
isolated from various fungal spp. that catalyzes the cyclization of  
farnesyl diphosphate (FPP) to trichodiene. The trichodiene synthase gene  
(Tox5) of F. sporotrichioides has previously been cloned and expressed as  
0.05-0.1% of total cell protein in E. coli. Polymerase chain reaction was  
used to amplify the trichodiene coding sequence carried on the plasmid  
pTS56-1. The resulting DNA, carrying a BamHI restriction site  
and the T7 gene 10 ribosome binding site and translational spacer element  
immediately upstream of the ATG start codon as well as a HindIII site  
adjacent to the translational stop codon, was inserted into the  
corresponding sites of the expression vector pLM1. The latter vector  
carried the promoter and translational leader sequence from T7 gene 10 and  
the E. coli rmBT1T2 tandem transcription terminator. This construct was  
cloned into E. coli BL21(DE3). The resulting transformants, when induced  
with iso-Pr .beta.-D-thiogalactoside, produced trichodiene synthase as  
20-30% of total sol. protein. The recombinant synthase, which could be  
purified 5-fold to homogeneity by (NH4)2SO4 pptn., ion-exchange chromatog.  
on Q Sepharose, and gel filtration on Superose 12, was identical to native  
protein in steady-state kinetic parameters and mobility on SDS-PAGE and  
had the expected MENFP N-terminal sequence.

L4 ANSWER 9 OF 19 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:759657 HCAPLUS  
DOCUMENT NUMBER: 123:220086  
TITLE: Accurate in vitro transcription from circularized  
plasmid templates by plant whole cell extracts  
AUTHOR(S): Zhu, Qun; Chappell, Joseph; Hedrick, Susan A.; Lamb,  
Chris  
CORPORATE SOURCE: Plant Biology Laboratory, Salk Institute Biological  
Studies, La Jolla, CA, 92037, USA  
SOURCE: Plant Journal (1995), 7(6), 1021-30  
CODEN: PLJUED; ISSN: 0960-7412  
PUBLISHER: Blackwell  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A convenient in vitro transcription system using monocot and dicot whole  
cell exts. and circular DNA templates has been developed. The  
system consists of incubating template and whole cell ext. to generate  
initiation complexes, followed by addn. of nucleotide triphosphates to  
support elongation, and primer extension assay to detect authentic  
transcripts. This in vitro transcription system required circularized  
templates and was essentially inactive with linearized templates.  
Accurate in vitro transcription of a rice phenylalanine ammonia-lyase  
(PAL) promoter-.beta.-glucuronidase (GUS) gene fusion and a tobacco  
**sesquiterpene cyclase** promoter-GUS gene fusion was  
examd. in their homologous whole cell exts., and the optimal concns. for  
several reaction components, including DNA template, whole cell  
ext., monovalent and divalent cations, were detd. for specific initiation  
from the in vivo start site. Transcription was inhibited by low concns.  
of .alpha.-amanitin, demonstrating that the reaction was mediated by RNA  
polymerase II. Accurate transcription initiation was dependent on the  
TATA-box motif within the resp. promoters. Based on the effect of delayed  
addn. of sarkosyl at a concn. sufficient to inhibit transcription  
initiation but not elongation, three to four rounds of transcription were

initiated in std. assays.

L4 ANSWER 10 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2003:131950 BIOSIS  
DOCUMENT NUMBER: PREV200300131950  
TITLE: Induction of a unique **sesquiterpene**  
**cyclase** by secondary signals released from  
pathogen-challenged cells.  
AUTHOR(S): Schoenbeck, Mark A. [Reprint Author]; Lusso, Marcos  
[Reprint Author]; Chappell, Joe [Reprint Author];  
Mandujano-Chavez, Alejandra  
CORPORATE SOURCE: Plant Physiology/Biochemistry/Molecular Biology Program,  
University of Kentucky, Lexington, KY, USA  
SOURCE: Plant Biology (Rockville), (1998) Vol. 1998, pp. 152.  
print.  
Meeting Info.: Annual Meeting of the American Society of  
Plant Physiologists combined with the 9th International  
Conference on Arabidopsis Research. Madison, WI, USA. June  
27-July 01, 1998. American Society of Plant Physiologists  
(ASPP).  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 12 Mar 2003  
Last Updated on STN: 12 Mar 2003

L4 ANSWER 11 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1996:14554 BIOSIS  
DOCUMENT NUMBER: PREV199698586689  
TITLE: Characterization of potato **sesquiterpene**  
**cyclase** cDNA clones.  
AUTHOR(S): Zook, M.  
CORPORATE SOURCE: Dep. Bot. Plant Pathol., Mich. State Univ., East Lansing,  
MI 48824, USA  
SOURCE: Phytopathology, (1995) Vol. 85, No. 10, pp. 1161.  
Meeting Info.: Annual Meeting of the American  
Phytopathological Association. Pittsburgh, Pennsylvania,  
USA. August 12-16, 1995.  
CODEN: PHYTAJ. ISSN: 0031-949X.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 4 Jan 1996  
Last Updated on STN: 4 Jan 1996

L4 ANSWER 12 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1994:142694 BIOSIS  
DOCUMENT NUMBER: PREV199497155694  
TITLE: Partial genomic sequence of potato **sesquiterpene**  
**cyclase**.  
AUTHOR(S): Zook, M.  
CORPORATE SOURCE: Dep. Botany and Plant Pathol., Michigan State Univ., East  
Lansing, MI 48824, USA  
SOURCE: Phytopathology, (1993) Vol. 83, No. 12, pp. 1382.  
Meeting Info.: Joint Meeting of the American  
Phytopathological Society and the Society of Nematologists  
on Plant Pathology Beyond 2000. Nashville, Tennessee, USA.  
November 6-10, 1993.  
CODEN: PHYTAJ. ISSN: 0031-949X.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 30 Mar 1994  
Last Updated on STN: 31 Mar 1994

L4 ANSWER 13 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
 ACCESSION NUMBER: 1992:405136 BIOSIS  
 DOCUMENT NUMBER: PREV199243061011; BR43:61011  
 TITLE: ISOLATION AND CHARACTERIZATION OF CDNA AND GENOMIC CLONES  
 ENCODING AN INDUCIBLE **SESQUITERPENE**  
**CYCLASE** FROM TOBACCO.  
 AUTHOR(S): FACCHINI P J [Reprint author]; CHAPPELL J  
 CORPORATE SOURCE: PLANT PHYSIOLOGY/BIOCHEMISTRY/MOLECULAR BIOLOGY PROGRAM,  
 AGRONOMY DEP, UNIVERSITY KENTUCKY, LEXINGTON, KY 40546, USA  
 SOURCE: Plant Physiology (Rockville), (1992) Vol. 99, No. 1 SUPPL,  
 pp. 86.  
 Meeting Info.: ANNUAL MEETING OF THE AMERICAN SOCIETY OF  
 PLANT PHYSIOLOGISTS, PITTSBURGH, PENNSYLVANIA, USA, AUGUST  
 1-5, 1992. PLANT PHYSIOL (BETHESDA).  
 CODEN: PLPHAY. ISSN: 0032-0889.  
 DOCUMENT TYPE: Conference; (Meeting)  
 FILE SEGMENT: BR  
 LANGUAGE: ENGLISH  
 ENTRY DATE: Entered STN: 26 Aug 1992  
 Last Updated on STN: 1 Oct 1992

L4 ANSWER 14 OF 19 AGRICOLA Compiled and distributed by the National  
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 (2004) on STN

ACCESSION NUMBER: 1999:22259 AGRICOLA  
 DOCUMENT NUMBER: IND21971988  
 TITLE: Cloning and bacterial expression of  
**sesquiterpene cyclase**, a key branch  
 point enzyme for the synthesis of sesquiterpenoid  
 phytoalexin capsidiol in UV-challenged leaves of  
 Capsicum annuum.  
 AUTHOR(S): Back, K.; He, S.; Kim, K.U.; Shin, D.H.  
 CORPORATE SOURCE: Chonnam National University, Kwangju, South Korea.  
 AVAILABILITY: DNAL (450 P699)  
 SOURCE: Plant and cell physiology, **Sept 1998**. Vol.  
 39, No. 9. p. 899-904  
 Publisher: Kyoto, Japan : Japanese Society of Plant  
 Physiologists.  
 CODEN: PCPHA5; ISSN: 0032-0781  
 NOTE: Includes references  
 PUB. COUNTRY: Japan  
 DOCUMENT TYPE: Article  
 FILE SEGMENT: Non-U.S. Imprint other than FAO  
 LANGUAGE: English

AB **Sesquiterpene cyclase**, a branch point enzyme in the  
 general isoprenoid pathway for the synthesis of phytoalexin capsidiol, was  
 induced in detached leaves of Capsicum annuum (pepper) by UV treatment.  
 The inducibility of cyclase enzyme activities paralleled the absolute  
 amount of cyclase protein(s) of pepper immunodetected by monoclonal  
 antibodies raised against tobacco **sesquiterpene cyclase**  
 . A cDNA library was constructed with poly(A)+ RNA isolated from 24 h  
 UV-challenged leaves of pepper. A cDNA clone for **sesquiterpene**  
**cyclase** in pepper was isolated by using a tobacco 5-epi  
 aristolochene synthase gene as a heterologous probe. The predicted protein  
 encoded by this cDNA was comprised of 559 amino acids and had a relative  
 molecular mass of 65,095. The primary structural information from the cDNA  
 clone revealed that it shared 77%, 72% and 49% identity with 5-epi  
 aristolochene, vetispiradiene, and cadinene synthase, respectively. The  
 enzymatic product catalyzed by the cDNA clone in bacteria was identified  
 as 5-epi aristolochene, as judged by argentation TLC. RNA blot  
 hybridization demonstrated the induction of an mRNA consistent with the  
 induction of cyclase enzyme activity in UV-treated pepper.

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ACCESSION NUMBER: 1998:49601 AGRICOLA  
DOCUMENT NUMBER: IND21378981  
TITLE: **Sesquiterpene cyclase** is not a determining factor for elicitor- and pathogen-induced capsidiol accumulation in tobacco.  
AUTHOR(S): Keller, H.; Czernic, P.; Ponchet, M.; Ducrot, P.H.; Back, K.; Chappell, J.; Ricci, P.; Marco, Y.  
AVAILABILITY: DNAL (450 P693)  
SOURCE: Planta, July 1998. Vol. 205, No. 3. p. 467-476  
Publisher: Berlin ; New York : Springer-Verlag, 1925-  
CODEN: PLANAB; ISSN: 0032-0935  
NOTE: Includes references  
PUB. COUNTRY: Germany  
DOCUMENT TYPE: Article  
FILE SEGMENT: Non-U.S. Imprint other than FAO  
LANGUAGE: English  
AB The induction of **sesquiterpene cyclase**, a key phytoalexin biosynthetic enzyme, and the accumulation of phytoalexins in relation to the induction of a hypersensitive response (HR) and cell necrosis in tobacco (*Nicotiana tabacum* L.) were investigated. When tobacco leaves were inoculated with virulent or avirulent isolates of *Ralstonia solanacearum*, steady-state levels of mRNA complementary to cDNA of the sensitivity-related (sts) gene str319 were dramatically induced. This cDNA clone is greater than 90% homologous with a gene coding for 5-epi-aristolochene synthase (EAS), previously described as a branch-point enzyme regulating the synthesis of capsidiol, the major sesquiterpenoid phytoalexin found in tobacco. Accumulation of EAS transcripts in leaves after inoculation with virulent and avirulent strains of *R. solanacearum*, or after treatment with necrotizing or non-necrotizing elicitors was rapid but transient, and restricted to the site of infiltration. Two highly similar **sesquiterpene cyclase** activities, 5-epi-aristolochene synthase and a vetispiradiene synthase-like activity, were found in extracts of elicitor-challenged and *R. solanacearum*-inoculated tobacco. Under all conditions tested, the induction of cyclase activity was closely correlated with induction of the cyclase mRNA level. In contrast, high levels of capsidiol were found only after treatment with the necrosis-inducing elicitor cryptogein, or after infiltration with HR-inducing bacterial strains. Low levels of capsidiol did accumulate after application of capsaicin, an elicitor that induces little or no necrosis on tobacco, or after infection with a virulent bacterium. Hence, capsidiol accumulation, not 5-epi-aristolochene synthase gene expression or total **sesquiterpene cyclase** enzyme activity, appears to be a good marker for the HR of tobacco.

L4 ANSWER 16 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1996:232423 BIOSIS  
DOCUMENT NUMBER: PREV199698796552  
TITLE: A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis.  
AUTHOR(S): Wildung, Mark R.; Croteau, Rodney [Reprint author]  
CORPORATE SOURCE: Inst. Biol. Chem., Washington State University, Pullman, WA 99164-6340, USA  
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 16, pp. 9201-9204.  
CODEN: JBCHA3. ISSN: 0021-9258.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
OTHER SOURCE: EMBL-U48796; Genbank-U48796  
ENTRY DATE: Entered STN: 28 May 1996  
Last Updated on STN: 11 Jul 1996  
AB The committed step of taxol (paclitaxel) biosynthesis is catalyzed by

taxa-4(5),11(12)-diene synthase, a diterpene cyclase responsible for transforming the ubiquitous isoprenoid intermediate geranylgeranyl diphosphate to the parent olefin with a taxane skeleton. To obtain the corresponding cDNA clone, a set of degenerate primers was constructed based on consensus sequences of related monoterpene, sesquiterpene, and diterpene cyclases. Two of these primers amplified 83-base pair fragment that was cyclase-like in sequence and that was employed as a hybridization probe to screen a cDNA library constructed from poly(A)<sup>+</sup> RNA extracted from Pacific yew (*Taxus brevifolia*) stems. Twelve independent clones with insert size in excess of 2 kilobase pairs were isolated and partially sequenced. One of these cDNA isolates was functionally expressed in *Escherichia coli*, yielding a protein that was catalytically active in converting geranylgeranyl diphosphate to a diterpene olefin that was confirmed to be taxa4(5),11(12)-diene by combined capillary gas chromatography-mass spectrometry. The sequence specifies an open reading frame of 2586 nucleotides, and the complete deduced polypeptide, including a long presumptive plastidial targeting peptide, contains 862 amino acid residues and has a molecular weight of 98,303, compared with about 79,000 previously determined for the mature native enzyme. Sequence comparisons with monoterpene, sesquiterpene, and diterpene cyclases of plant origin indicate a significant degree of similarity between these enzymes; the taxadiene synthase most closely resembles (46% identity, 67% similarity) abietadiene synthase, a diterpene cyclase from grand fir.

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ACCESSION NUMBER: 96:53030 AGRICOLA  
DOCUMENT NUMBER: IND20530195  
TITLE: Identifying functional domains within terpene cyclases using a domain-swapping strategy.  
AUTHOR(S): Back, K.; Chappell, J.  
CORPORATE SOURCE: University of Kentucky, Lexington, KY.  
AVAILABILITY: DNAL (500 N21P)  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, June 25, 1996.  
Vol. 93, No. 13. p. 6841-6845  
Publisher: Washington, D.C. : National Academy of Sciences,  
CODEN: PNASA6; ISSN: 0027-8424  
NOTE: Includes references  
PUB. COUNTRY: District of Columbia; United States  
DOCUMENT TYPE: Article; Conference  
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension  
LANGUAGE: English

AB Cyclic terpenes and terpenoids are found throughout nature. They comprise an especially important class of compounds from plants that mediate plant-environment interactions, and they serve as pharmaceutical agents with antimicrobial and anti-tumor activities. Molecular comparisons of several terpene cyclases, the key enzymes responsible for the multistep cyclization of C<sub>10</sub>, C<sub>15</sub>, and C<sub>20</sub> allylic diphosphate substrates, have revealed a striking level of sequence similarity and conservation of exon position and size within the genes. Functional domains responsible for a terminal enzymatic step were identified by swapping regions approximating exons between a *Nicotiana tabacum* 5-epiaristolochene synthase (TEAS) gene and a *Hyoscyamus muticus* vetispiradiene synthase (HVS) gene and by characterization of the resulting chimeric enzymes expressed in bacteria. While exon 4 of the TEAS gene conferred specificity for the predominant reaction products of the tobacco enzyme, exon 6 of the HVS gene conferred specificity for the predominant reaction products of the *Hyoscyamus* enzyme. Combining these two functional domains of the TEAS and HVS genes resulted in a novel enzyme capable of synthesizing reaction products reflective of both parent enzymes. The relative ratio of the TEAS and HVS reaction products was also influenced by the source of exon 5 present in



the new chimeric enzymes. The association of catalytic activities with conserved but separate exonic domains suggests a general means for generating additional novel terpene cyclases.

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ACCESSION NUMBER: 1999:8743 AGRICOLA  
DOCUMENT NUMBER: IND21961068  
TITLE: Germacrene C synthase from *Lycopersicon esculentum* cv. VFNT Cherry tomato: cDNA isolation, characterization, and bacterial expression of the multiple product **sesquiterpene cyclase**.  
AUTHOR(S): Colby, S.M.; Crock, J.; Dowdle-Rizzo, B.; Lemaux, P.G.; Croteau, R.  
CORPORATE SOURCE: University of California, Berkeley, CA.  
AVAILABILITY: DNAL (500 N21P)  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, **Mar 3, 1998**. Vol. 95, No. 5. p. 2216-2221  
Publisher: Washington, D.C. : National Academy of Sciences,  
CODEN: PNASA6; ISSN: 0027-8424  
NOTE: Includes references  
PUB. COUNTRY: District of Columbia; United States  
DOCUMENT TYPE: Article; Conference  
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension  
LANGUAGE: English

AB Germacrene C was found by GC-MS and NMR analysis to be the most abundant sesquiterpene in the leaf oil of *Lycopersicon esculentum* cv. VFNT Cherry, with lesser amounts of germacrene A, guaia-6,9-diene, germacrene B, beta-caryophyllene, alpha-humulene, and germacrene D. Soluble enzyme preparations from leaves catalyzed the divalent metal ion-dependent cyclization of [1-3H]farnesyl diphosphate to these same sesquiterpene olefins, as determined by radio-GC. To obtain a germacrene synthase cDNA, a set of degenerate primers was constructed based on conserved amino acid sequences of related terpenoid cyclases. With cDNA prepared from leaf epidermis-enriched mRNA, these primers amplified a 767-bp fragment that was used as a hybridization probe to screen the cDNA library. Thirty-one clones were evaluated for functional expression of terpenoid cyclase activity in *Escherichia coli* by using labeled geranyl, farnesyl, and geranylgeranyl diphosphates as substrates. Nine cDNA isolates expressed sesquiterpene synthase activity, and GC-MS analysis of the products identified germacrene C with smaller amounts of germacrene A, B, and D. None of the expressed proteins was active with geranylgeranyl diphosphate; however, one truncated protein converted geranyl diphosphate to the monoterpene limonene. The cDNA inserts specify a deduced polypeptide of 548 amino acids (M(r) = 64,114), and sequence comparison with other plant sesquiterpene cyclases indicates that germacrene C synthase most closely resembles cotton delta-cadinene synthase (50% identity).

L4 ANSWER 19 OF 19 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN

ACCESSION NUMBER: 94:24633 AGRICOLA  
DOCUMENT NUMBER: IND20380755  
TITLE: 4S-limonene synthase from the oil glands of spearmint (*Mentha spicata*): cDNA isolation, characterization, and bacterial expression of the catalytically active monoterpene cyclase.  
AUTHOR(S): Colby, S.M.; Alonso, W.R.; Katahira, E.J.; McGarvey, D.J.; Croteau, R.  
AVAILABILITY: DNAL (381 J824)

SOURCE: The Journal of biological chemistry, Nov 5,  
1993. Vol. 268, No. 31. p. 23016-23024  
Publisher: Baltimore, Md. : American Society for  
Biochemistry and Molecular Biology.  
CODEN: JBCHA3; ISSN: 0021-9258

NOTE: Includes references  
PUB. COUNTRY: Maryland; United States  
DOCUMENT TYPE: Article  
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension  
LANGUAGE: English

AB The committed step in the biosynthesis of monoterpenes in mint (*Mentha*) species is the cyclization of geranyl pyrophosphate to the olefin (-)-4S-limonene catalyzed by limonene synthase (cyclase). Internal amino acid sequences of the purified enzyme from spearmint oil glands were utilized to design three distinct oligonucleotide probes. These probes were subsequently employed to screen a spearmint leaf cDNA library, and four clones were isolated. Three of these cDNA isolates were full-length and were functionally expressed in *Escherichia coli*, yielding a peptide that is immunologically recognized by polyclonal antibodies raised against the purified limonene synthase from spearmint and that is catalytically active in generating from geranyl pyrophosphate a product distribution identical to that of the native enzyme (principally limonene with small amounts of the coproducts alpha- and beta-pinene and myrcene). The longest open reading frame is 1800 nucleotides and the deduced amino acid sequence contains a putative plastidial transit peptide of approximately 90 amino acids and a mature protein of about 510 residues corresponding to the native enzyme. Several nucleotide differences in the 5'-untranslated region of all three full-length clones suggest the presence of several limonene synthase genes and/or alleles in the allotetraploid spearmint genome. Sequence comparisons with a **sesquiterpene cyclase**, epi-aristolochene synthase from tobacco, and a diterpene cyclase, casbene synthase from castor bean, demonstrated a significant degree of similarity between these three terpenoid cyclase types, the first three examples of this large family of catalysts to be described from higher plants.

=> s (amorpha-4,11-diene synthase or amorphadiene synthase) and (dna or rna or nucleic acid)  
1 FILES SEARCHED...

L6 9 (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHADIENE SYNTHASE) AND (DNA  
OR RNA OR NUCLEIC ACID)

=> dup rem l6

PROCESSING COMPLETED FOR L6

L7 6 DUP REM L6 (3 DUPLICATES REMOVED)

=> d l7 1-6 ibib ab

L7 ANSWER 1 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:18837 HCAPLUS

DOCUMENT NUMBER: 140:92683

TITLE: Preparation of amorpha-4,11-diene with transgenic  
microorganisms producing isopentenyl- and  
dimethylallyl pyrophosphates

INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas;  
Withers, Sydnor T.; Newman, Jack

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 75 pp., Cont.-in-part of U.S.  
Ser. No. 6,909.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004005678	A1	20040108	US 2003-411066	20030409
US 2003148479	A1	20030807	US 2001-6909	20011206

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206

AB Methods for synthesizing amorpha-4,11-diene from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous **nucleic acid** sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. Amorpha-4,11-diene is then produced with the transgenic microorganism which is further transformed with an optimized **amorpha-4,11-diene synthase** gene. The amorpha-4,11-diene may be used in synthesis of the antimalarial drug artemisinin. Thus, amorpha-4,11-diene was prepd. from mevalonate supplied in the medium with Escherichia coli transformed with plasmid pBBRMDIS-2, contg. the yeast genes idi (for isopentenyl pyrophosphate isomerase) and ispA (for farnesyl pyrophosphate synthase) and the genes for mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and **amorpha-4,11-diene synthase**. The yield was 2 .mu.g amorpha-4,11-diene/mL.

L7 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:609986 HCAPLUS

DOCUMENT NUMBER: 139:160786

TITLE: Biosynthesis of isopentenyl pyrophosphate using recombinant microbial metabolic pathways

INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim, Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo; Newman, Jack; Khlebnikov, Artem Valentinovich

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 40 pp.  
CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003148479	A1	20030807	US 2001-6909	20011206
US 2004005678	A1	20040108	US 2003-411066	20030409

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206

AB Methods for synthesizing isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous **nucleic acid** sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous **nucleic acid** sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides **nucleic acid** sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

L7 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2003-18363 BIOTECHDS

TITLE: Engineering a mevalonate pathway in Escherichia coli for production of terpenoids;  
vector-mediated gene transfer and expression in host cell for strain improvement and terpene preparation

AUTHOR: MARTIN VJJ; PITERA DJ; WITHERS ST; NEWMAN JD; KEASLING JD

CORPORATE SOURCE: Univ Calif Berkeley; Lawrence Berkeley Natl Lab

LOCATION: Keasling JD, Univ Calif Berkeley, Dept Chem Engrg, 201 Gilman Hall, Berkeley, CA 94720 USA

SOURCE: NATURE BIOTECHNOLOGY; (2003) 21, 7, 796-802  
ISSN: 1087-0156

DOCUMENT TYPE: Journal

LANGUAGE: English

AB AUTHOR ABSTRACT - Isoprenoids are the most numerous and structurally diverse family of natural products. Terpenoids, a class of isoprenoids often isolated from plants, are used as commercial flavor and fragrance compounds and antimalarial or anticancer drugs. Because plant tissue extractions typically yield low terpenoid concentrations, we sought an alternative method to produce high-value terpenoid compounds, such as the antimalarial drug artemisinin, in a microbial host. We engineered the expression of a synthetic amorpho- 4,11-diene synthase gene and the mevalonate isoprenoid pathway from *Saccharomyces cerevisiae* in *Escherichia coli*. Concentrations of amorphadiene, the sesquiterpene olefin precursor to artemisinin, reached 24 µg caryophyllene equivalent/ml. Because isopentenyl and dimethylallyl pyrophosphates are the universal precursors to all isoprenoids, the strains developed in this study can serve as platform hosts for the production of any terpenoid compound for which a terpene synthase gene is available. (7 pages)

L7 ANSWER 4 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN DUPLICATE 1

ACCESSION NUMBER: 2001:63594 AGRICOLA

DOCUMENT NUMBER: IND23222235

TITLE: **Amorpha-4,11-diene synthase**: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin.

AUTHOR(S): Wallaart, T.E.; Bouwmeester, H.J.; Hille, J.; Poppinga, L.; Maijers, N.C.A.

AVAILABILITY: DNAL (450 P693)

SOURCE: Planta, Feb 2001. Vol. 212, No. 3. p. 460-465  
Publisher: Berlin ; New York : Springer-Verlag, 1925-  
CODEN: PLANAB; ISSN: 0032-0935

NOTE: Includes references

PUB. COUNTRY: Germany

DOCUMENT TYPE: Article

FILE SEGMENT: Non-U.S. Imprint other than FAO

LANGUAGE: English

AB The sesquiterpenoid artemisinin, isolated from the plant *Artemisia annua* L., and its semi-synthetic derivatives are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compound is the cyclisation of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely amorpha-4,11-diene. Here we describe the isolation of a cDNA clone encoding **amorpha-4,11-diene synthase**. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of *A. annua*. When expressed in *Escherichia coli*, the recombinant enzyme catalyses the formation of amorpha-4,11-diene from farnesyl diphosphate. Introduction of the gene into tobacco (*Nicotiana tabacum* L.) resulted in the expression of an active enzyme and the accumulation of amorpha-4,11-diene ranging from 0.2 to 1.7 ng per g fresh weight.

L7 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:144616 HCAPLUS

DOCUMENT NUMBER: 132:204840

TITLE: *Artemisia annua* **amorpha-4,11-diene synthase**, its cDNA, recombinant expression, and methods of amorpha-4,11-diene and artemisinin synthesis via

transgenic plants  
 INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan  
 PATENT ASSIGNEE(S): Neth.  
 SOURCE: Eur. Pat. Appl., 41 pp.  
 CODEN: EPXXDW  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9957423	A1	20000321	AU 1999-57423	19990827
AU 766764	B2	20031023		
EP 1108041	A2	20010620	EP 1999-944535	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9913196	A	20010925	BR 1999-13196	19990827
JP 2002523101	T2	20020730	JP 2000-567711	19990827
ZA 2001001455	A	20010828	ZA 2001-1455	20010221
PRIORITY APPLN. INFO.:			EP 1998-202854	A 19980827
			WO 1999-EP6302	W 19990827

AB **Amorpha-4,11-diene synthase** from *Artemisia annua* L., its cDNA, recombinant expression, and methods of prep. amorpha-4,11-diene and artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. Amorpha-4,11-diene is a precursor of the new anti-malarial drug artemisinin produced by the plant *Artemisia annua* L. A cDNA encoding **amorpha-4,11-diene synthase** from *A. annua* has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4,11-diene synthase** expressed in *E. coli*, transgenic tobacco, and transgenic *A. annua* catalyzed conversion of FPP into amorpha-4,11-diene. Further conversion of amorpha-4,11-diene into artemisinin was obsd. in transgenic *A. annua*. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2  
 ACCESSION NUMBER: 2000:826620 HCAPLUS  
 DOCUMENT NUMBER: 134:189822  
 TITLE: **Amorpha-4,11-diene Synthase** of *Artemisia annua*:  
 cDNA Isolation and Bacterial Expression of a Terpene Synthase Involved in Artemisinin Biosynthesis  
 AUTHOR(S): Chang, Yung-Jin; Song, Seung-Hwan; Park, Si-Hyung; Kim, Soo-Un  
 CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, 441-744, S. Korea

SOURCE: Archives of Biochemistry and Biophysics (2000),  
 383(2), 178-184  
 CODEN: ABBIA4; ISSN: 0003-9861

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4,11-diene synthase** for metabolic engineering. PCR amplification of genomic **DNA** with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp **DNA** fragment. This fragment was used in Northern blot anal. as a probe, showing approx. 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A sol. fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS anal. as amorpha-4,11-diene. (c) 2000 Academic Press.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> file registry		
COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	93.36	93.57
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL
	ENTRY	SESSION
CA SUBSCRIBER PRICE	-8.32	-8.32

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STRUCTURE FILE UPDATES: 7 JUN 2004 HIGHEST RN 690625-61-7  
 DICTIONARY FILE UPDATES: 7 JUN 2004 HIGHEST RN 690625-61-7

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 6, 2004

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Experimental and calculated property data are now available. For more information enter HELP PROP at an arrow prompt in the file or refer to the file summary sheet on the web at:  
<http://www.cas.org/ONLINE/DBSS/registryss.html>

=> s sesquiterpene cyclase  
 69 SESQUITERPENE  
 2788 CYCLASE  
 L8 10 SESQUITERPENE CYCLASE  
 (SESQUITERPENE (W) CYCLASE)

=> d 18

L8 ANSWER 1 OF 10 REGISTRY COPYRIGHT 2004 ACS on STN  
RN 463369-40-6 REGISTRY  
CN Cyclase, farnesyl pyrophosphate (corn strain McC gene stc1) (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN GenBank AAK73113  
CN GenBank AAK73113 (Translated from: GenBank AF391808)  
CN **Sesquiterpene cyclase (corn strain McC chromosome 9 gene stc1)**  
FS PROTEIN SEQUENCE  
DR 467473-02-5  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
2 REFERENCES IN FILE CA (1907 TO DATE)  
2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> s amorpho-4, 11-diene synthase  
28 AMORPHA  
13759876 4  
873460 11  
210105 DIENE  
25230 SYNTHASE  
L9 6 AMORPHA-4, 11-DIENE SYNTHASE  
(AMORPHA (W) 4 (W) 11 (W) DIENE (W) SYNTHASE)

=> d 19

L9 ANSWER 1 OF 6 REGISTRY COPYRIGHT 2004 ACS on STN  
RN 642550-56-9 REGISTRY  
CN **DNA (synthetic Saccharomyces cerevisiae amorpho-4,11-diene synthase gene) (9CI) (CA INDEX NAME)**  
OTHER NAMES:  
CN 37: PN: US20040005678 SEQID: 37 claimed DNA  
FS NUCLEIC ACID SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, USPATFULL  
DT.CA Caplus document type: Patent  
RL.P Roles from patents: BIOL (Biological study); PRP (Properties); USES (Uses)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> s amorphadiene synthase  
1 AMORPHADIENE  
25230 SYNTHASE  
L10 0 AMORPHADIENE SYNTHASE  
(AMORPHADIENE (W) SYNTHASE)

=> d his

(FILE 'HOME' ENTERED AT 11:18:59 ON 09 JUN 2004)

FILE 'HCAPLUS, AGRICOLA, BIOSIS, BIOTECHDS' ENTERED AT 11:19:39 ON 09 JUN 2004

L1	59 S SESQUITERPENE CYCLASE AND DNA
L2	40 DUP REM L1 (19 DUPLICATES REMOVED)
L3	19 S L2 AND 1990-1998/PY
L4	19 FOCUS L3 1-
L5	0 S L4 AND ARTEMISIA ANNUA
L6	9 S (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHADIENE SYNTHASE) AND (D
L7	6 DUP REM L6 (3 DUPLICATES REMOVED)

FILE 'REGISTRY' ENTERED AT 11:30:40 ON 09 JUN 2004

L8	10 S SESQUITERPENE CYCLASE
L9	6 S AMORPHA-4, 11-DIENE SYNTHASE
L10	0 S AMORPHADIENE SYNTHASE

=> log y

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	45.09	138.66
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL
	ENTRY	SESSION
CA SUBSCRIBER PRICE	0.00	-8.32

STN INTERNATIONAL LOGOFF AT 11:33:12 ON 09 JUN 2004



For IFW

=> file hcaplus medline biosis agricola scisearch biotechds  
COST IN U.S. DOLLARS

SINCE FILE  
ENTRY  
0.21

TOTAL  
SESSION  
0.21

FULL ESTIMATED COST

FILE 'HCAPLUS' ENTERED AT 12:32:16 ON 09 JUN 2004  
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FILE 'BIOSIS' ENTERED AT 12:32:16 ON 09 JUN 2004  
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FILE 'AGRICOLA' ENTERED AT 12:32:16 ON 09 JUN 2004

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FILE 'BIOTECHDS' ENTERED AT 12:32:16 ON 09 JUN 2004  
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=> s (Amorpha-4,11-diene Synthase or Amorpha-4,11-diene Synthetase)  
L1 34 (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHA-4,11-DIENE SYNTHETASE)

=> dup rem l1  
PROCESSING COMPLETED FOR L1  
L2 13 DUP REM L1 (21 DUPLICATES REMOVED)

=> s l2 and dna  
L3 3 L2 AND DNA

=> d l3 1-3 ibib ab

L3 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 2003:609986 HCAPLUS  
DOCUMENT NUMBER: 139:160786  
TITLE: Biosynthesis of isopentenyl pyrophosphate using  
recombinant microbial metabolic pathways  
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim,  
Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo;  
Newman, Jack; Khlebnikov, Artem Valentinovich  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S. Pat. Appl. Publ., 40 pp.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003148479	A1	20030807	US 2001-6909	20011206
US 2004005678	A1	20040108	US 2003-411066	20030409

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206

AB Methods for synthesizing isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous nucleic acid sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides nucleic acid sequences, enzymes, expression

vectors, and transformed host cells for carrying out the methods.

L3 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 2000:826620 HCAPLUS  
DOCUMENT NUMBER: 134:189822  
TITLE: **Amorpha-4,11-diene Synthase** of *Artemisia annua*:  
cDNA Isolation and Bacterial Expression of a Terpene  
Synthase Involved in Artemisinin Biosynthesis  
AUTHOR(S): Chang, Yung-Jin; Song, Seung-Hwan; Park, Si-Hyung;  
Kim, Soo-Un  
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research  
Center for New Biomaterials in Agriculture, Seoul  
National University, Suwon, 441-744, S. Korea  
SOURCE: Archives of Biochemistry and Biophysics (2000),  
383(2), 178-184  
CODEN: ABBIA4; ISSN: 0003-9861  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB *Artemisia annua*, an indigenous plant to Korea, contains an antimalarial  
sesquiterpene, artemisinin. The first committed step of artemisinin  
biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene  
synthase to produce an amorphane-type ring system. The aims of this  
research were to molecularly clone and express **amorpha-4**  
**,11-diene synthase** for metabolic  
engineering. PCR amplification of genomic DNA with a pair of  
primers, designed from the conserved regions of sesquiterpene synthases of  
several plants, produced a 184-bp DNA fragment. This fragment  
was used in Northern blot anal. as a probe, showing approx. 2.2 kb of a  
single band. Its sequence information was used to produce 2106 bp of a  
full-length cDNA sequence including 1641 bp of open reading frame for 546  
amino acids (kcs12) through a rapid amplification of cDNA ends (RACE).  
The deduced amino acid sequence displayed 36% identity with  
5-epi-aristolochene synthase of *Nicotiana tabacum*. A sol. fraction of  
*Escherichia coli* harboring kcs12 catalyzed the cyclization of farnesyl  
diphosphate to produce a sesquiterpene, which was identified through GC-MS  
anal. as amorpha-4,11-diene. (c) 2000 Academic Press.  
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 2000:144616 HCAPLUS  
DOCUMENT NUMBER: 132:204840  
TITLE: *Artemisia annua* **amorpha-4**,  
**11-diene synthase**, its  
cDNA, recombinant expression, and methods of  
amorpha-4,11-diene and artemisinin synthesis via  
transgenic plants  
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan  
PATENT ASSIGNEE(S): Neth.  
SOURCE: Eur. Pat. Appl., 41 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,  
 CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,  
 IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD,  
 MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,  
 SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY,  
 KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,  
 ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,  
 CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9957423 A1 20000321 AU 1999-57423 19990827

AU 766764 B2 20031023

EP 1108041 A2 20010620 EP 1999-944535 19990827

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
 IE, SI, LT, LV, FI, RO

BR 9913196 A 20010925 BR 1999-13196 19990827

JP 2002523101 T2 20020730 JP 2000-567711 19990827

ZA 2001001455 A 20010828 ZA 2001-1455 20010221

PRIORITY APPLN. INFO.:

EP 1998-202854 A 19980827

WO 1999-EP6302 W 19990827

AB **Amorpha-4,11-diene**

**synthase** from *Artemisia annua* L., its cDNA, recombinant expression, and methods of prep. amorpha-4,11-diene and artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. Amorpha-4,11-diene is a precursor of the new anti-malarial drug artemisinin produced by the plant *Artemisia annua* L. A cDNA encoding **amorpha-4,11-diene synthase** from *A. annua* has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4,11-diene synthase** expressed in *E. coli*, transgenic tobacco, and transgenic *A. annua* catalyzed conversion of FPP into amorpha-4,11-diene. Further conversion of amorpha-4,11-diene into artemisinin was obsd. in transgenic *A. annua*. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s Amorpha-4,11-diene Synthetase

L4 0 AMORPHA-4,11-DIENE SYNTHETASE

=> s Amorpha-4,11-diene Synth?

3 FILES SEARCHED...

L5 34 AMORPHA-4,11-DIENE SYNTH?

=> d his

(FILE 'HOME' ENTERED AT 12:31:40 ON 09 JUN 2004)

FILE 'HCAPLUS, MEDLINE, BIOSIS, AGRICOLA, SCISEARCH, BIOTECHDS' ENTERED AT 12:32:16 ON 09 JUN 2004

L1 34 S (AMORPHA-4,11-DIENE SYNTHASE OR AMORPHA-4,11-DIENE SYNTHETASE

L2 13 DUP REM L1 (21 DUPLICATES REMOVED)

L3 3 S L2 AND DNA

L4 0 S AMORPHA-4,11-DIENE SYNTHETASE

L5 34 S AMORPHA-4,11-DIENE SYNTH?

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

15.84

16.05

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE

TOTAL

ENTRY

SESSION

CA SUBSCRIBER PRICE

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-2.08

STN INTERNATIONAL LOGOFF AT 12:34:54 ON 09 JUN 2004